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Adult Mice as a Model for Early Onset Group B Streptococcal Disease

D. E. WENNERSTROM* AND R. W. SCHUTT

Department of Microbiology and Immunology, The University of Arkansas for Medical Sciences, Little Rock, Arkansas 72201

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The intravenous inoculation of adult mice with virulent group B streptococci serotype Ia resulted in fulminating sepsis with extensive colonization of the lungs and kidneys. The time course of the infection lasting 24 to 40 h, extensive pulmonary colonization, and resistance of the type Ia organism to phagocytosis in the absence of specific antibody suggest that mice are an appropriate model for the study of early onset streptococcal infection of human neonates.

Group B streptococci are recognized as major etiological agents causing infection of the newborn. One clinical syndrome produced by these organisms has been described as early onset infection, which is characterized by a rapidly fulminant sepsis with symptoms of respiratory distress and shock during the first 24 h after birth (3). It has a mortality rate that approaches 100% and is accompanied by significant pathological changes of the lungs, which include interstitial hemorrhage, pneumonia, pulmonary congestion, and intraalveolar gram-positive cocci (1, 3, 5).

Serotype Ia is the predominant organism causing the early onset syndrome (3). Klesius et al. have found that type Ia is seldom attacked by polymorphonuclear leukocytes from humans, baboons, or chimpanzees (6). Further, Mathews et al. have described a specific opsonizing antibody, which is required for the opsonization of type Ia, whereas the other types of group B streptococci were opsonized nonspecifically by human plasma (8). These investigations have suggested that fatal sepsis results when an exposed infant lacks phagocytic activity, opsonizing antibody, or type-specific agglutinins.

An animal model is needed for the systematic investigation of those factors of both the host and group B streptococci, which are determinants of the ability of these organisms to cause disease. The animal models that have been proposed have required exposure to large numbers of group B streptococci administered as aerosols to infant rats (T. Y. Sabet, P. L. Kelly, and E. N. Fox, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, B66, p. 26), rabbits (M. Sherman, E. Goldstein, W. Lippert, and R. Wennberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, B67, p. 26), or intravenously to adult mice (4). However, the organisms either failed to initiate a progressive infection or they initially decreased in the tissues. Therefore, it seemed reasonable to investigate the infection of mice by type Ia because Lancefield et al. have demonstrated that the type Ia organism can be made highly virulent for these animals (7).

Group B streptococci types Ia and III were a generous gift from R. C. Lancefield. Each organism was grown in Todd-Hewitt broth and then passaged 10 times in male BALB/c mice weighing 20 to 25 g, as described by Lancefield et al. (7). The subsequent intraperitoneal 50% lethal doses of the Ia and III types in 0.5 ml of saline were 17.0×10^0 colony-forming units (CFU) and 1.9×10^8 CFU, respectively.

Figure 1 shows the growth curve of the virulent type Ia in the blood of five mice after intravenous inoculation with 0.2 ml of saline containing 670 CFU from an exponential-phase culture. The infection was monitored by sampling 100 or 20 μl of blood from the tail vein and plating according to standard methods. The sepsis was rapidly fulminant, reaching a maximum amount of 9.6×10^4 CFU/0.1 ml of blood at 16 h and remaining near 1×10^5 CFU/0.1 ml thereafter. Symptoms of the infection characterized by lethargy and labored breathing appeared at 16 h and progressed until 28 h when the animals were moribund. A larger dose of 2.8×10^3 CFU killed four of five mice within 24 h after intraperitoneal challenge.

To determine the effect of the sepsis on the colonization of the mouse tissue, groups of five mice each were killed at selected hours after intravenous challenge with 670 to 690 CFU. Spleens, livers, kidneys, and lungs were aseptically removed, weighed, and homogenized with a mortar and pestle, and the number of CFU was determined by plating (Table 1). The number of streptococci per 0.1 g of tissue (wet weight)

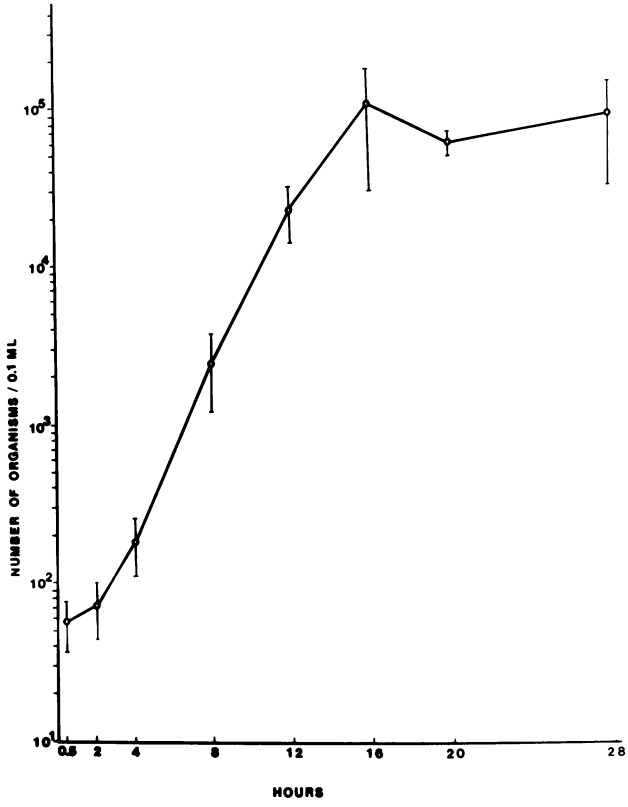

FIG. 1. *Time course of proliferation of type Ia in the blood of BALB/c mice sampled at various times after intravenous inoculation with 670 CFU/0.2 ml. Each point is the mean number of CFU from five mice ± the standard deviation.*

TABLE 1. *Growth in vivo of group B streptococci in five mouse tissues*

Time[a] (h)	No. of streptococci[b]				
	Blood	Liver	Spleen	Lung	Kidney
0.5	$6.9 \times 10^1 \pm 1.6$	$6.3 \times 10^1 \pm 0.5$	$2.2 \times 10^2 \pm 0.7$	$1.1 \times 10^2 \pm 0.5$	$2.5 \times 10^1 \pm 0.9$
4	$3.6 \times 10^1 \pm 1.3$	$3.6 \times 10^1 \pm 1.7$	$2.5 \times 10^2 \pm 0.7$	$4.6 \times 10^1 \pm 3.5$	$2.6 \times 10^1 \pm 1.3$
8	$1.8 \times 10^2 \pm 0.4$	$1.0 \times 10^2 \pm 0.3$	$8.5 \times 10^2 \pm 2.2$	$2.9 \times 10^2 \pm 1.0$	$7.6 \times 10^1 \pm 1.6$
28[c]	$8.0 \times 10^4 \pm 5.2$	$2.7 \times 10^4 \pm 0.1$	$7.3 \times 10^4 \pm 6.1$	$6.7 \times 10^5 \pm 0.7$	$3.8 \times 10^5 \pm 0.2$
37	$1.1 \times 10^5 \pm 0.5$	$8.9 \times 10^4 \pm 2.71$	$1.3 \times 10^5 \pm 1.45$	$5.2 \times 10^6 \pm 1.14$	$3.2 \times 10^6 \pm 3.3$

[a] A 0.2-ml portion of saline containing 6.9×10^2 organisms was injected into a lateral tail vein at zero time.
[b] Each value is the mean number of streptococci per 0.1 ml of blood or 0.1 g of organ from five mice ± the standard deviation.
[c] Animals for this time were inoculated in a separate experiment (Fig. 1) and received 6.7×10^2/0.2 ml.

increased with time in all tissues sampled. The spleen and lungs were prominently colonized 30 min after challenge, whereas the lungs and kidneys contained a log greater number of organisms than the other tissues late in the infection.

The involvement of each tissue in the infection is more easily seen in Table 2, which presents for each time after challenge (Table 1) the number of bacteria in a tissue as a percentage of that obtained from the five tissues tested at that time. It is apparent that the relative colonization

TABLE 2. *Distribution[a] in vivo of virulent group B streptococci in five mouse tissues*

Time (h)	Group B streptococci (%)				
	Blood	Liver	Spleen	Lung	Kidney
0.5	14.0	12.7	45.0	23.2	5.1
4	9.2	9.1	63.4	11.6	6.6
8	11.8	6.9	56.9	19.2	5.1
28	6.5	2.2	6.0	54.2	31.1
37	1.2	1.0	1.5	60.0	36.2

[a] Computed from the data in Table 1.

TABLE 3. *Phagocytosis of virulent and avirulent strains of group B streptococci by mouse peritoneal macrophages*

Streptococcus type	Antiserum to Ia	Phagocytosis[a] (cpm)	% cpm phagocytized	No. of organisms[b] phagocytized
Ia[c]	−	124.8 ± 17.2	0.6	4.1×10^4
Ia	+	429.1 ± 141.0	1.9	1.4×10^5
III[d]	−	2569.8 ± 216.8	7.7	8.1×10^5

[a] The mean number of counts per minute of triplicate samples ± the standard deviation after a 60-min incubation.

[b] The product of the specific activity of the organisms (CFU/counts per minute) and the number of counts per minute retained in the vials after subtraction of background counts per minute (49 ± 5.6).

[c] 7.3×10^6 CFU containing 2.2×10^4 cpm.

[d] 1.0×10^7 CFU containing 3.4×10^4 cpm.

of blood, liver, and spleen decreased, whereas that of the lungs and kidneys increased with progressing infection. These results suggest that the lungs and kidneys are primary sites of involvement in the fulminating sepsis by the virulent type Ia organism.

The growth of the bacteria in the blood and organs after challenge with 40 50% lethal doses (690 CFU) suggested that the type Ia organism evaded the cellular defense mechanisms of the mice. The ability of mouse cells to phagocytize the organism was tested quantitatively by incubating radioactively labeled streptococci with macrophages that had been isolated from the peritoneal cavity (Table 3). The organisms were labeled by incubation of 6×10^7 CFU for 3 h with 10 μCi of [1-^{14}C]sodium oleate (Amersham/Searle, specific activity 51 mCi/mmol) complexed to 2 mg/ml of fatty acid-free bovine serum albumin (Miles Laboratories) (9). Normal macrophages (3 ml from 6 mice) were obtained by adding to the peritoneal cavity Earle balanced salt solution (Pacific Biologicals) containing added $CaCl_2$ (1.8 mM), $MgSO_4$ (0.8 mM), glutamine (2 mM), minimal essential medium amino acids (Grand Island Biological Co.), and 10% newborn calf serum (GIBCO) that had been heat-inactivated at 56°C for 30 min. A 1-ml portion of a 1:8 dilution in medium was added to scintillation vials, and the cells were allowed to attach for 60 min at 37°C. The medium was then decanted, and 1 ml of fresh medium was added followed by 0.2 ml of labeled streptococci in saline containing 2.2×10^4 cpm (type Ia) or 3.4×10^4 cpm (type III). The vials were incubated for 60 min at 37°C. Macrophages attached to coverslips and stained with Giemsa were observed to contain intracellular streptococci after incubation in Leighton tubes under similar conditions. Radioactivity was determined in the washed phagocytes by counting in Bray solution (2). Background counts (mean 49 ± 5.6 standard deviation) were those retained in a set of unincubated vials. Table 3 shows that the virulent type Ia was minimally attacked, whereas the avirulent type III was phagocytized to a much greater extent. The addition of 0.2 ml of antiserum to the incubation medium containing type Ia increased phagocytosis by threefold. The antiserum was raised in BALB/c mice by an intraperitoneal injection each week for 3 weeks with 0.5 ml of saline containing 5×10^8 CFU that had been killed by heating to 80°C for 1 h. Antiserum was collected 10 days after the last injection and agglutinated type Ia, but not type III, organisms when diluted 1:10. Passive immunization of mice with 0.2 ml of undiluted antiserum intravenously 30 min before intravenous challenge with 670 CFU was completely protective. The same volume of injected antiserum diluted 1:10 protected four of five mice. These results show the importance of antibody for phagocytic activity by mouse cells and subsequent protection of the animal from infection by the Ia organism.

In this study, the infection of adult mice by virulent group B streptococci type Ia has been found to be markedly similar to group B early onset neonatal disease in the time course of infection, principal organ involvement, and requirement for specific antibody for the opsonization and phagocytosis of the organism. These results suggest that the mouse is a good model for the study of the infectious process of this organism.

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